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Background

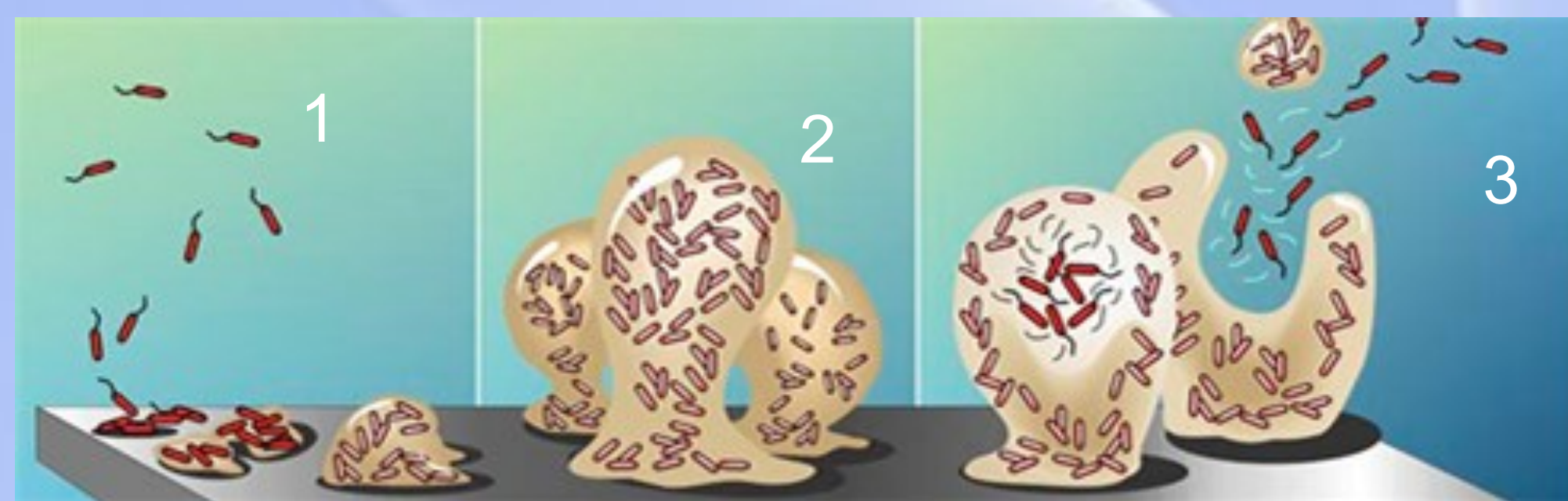


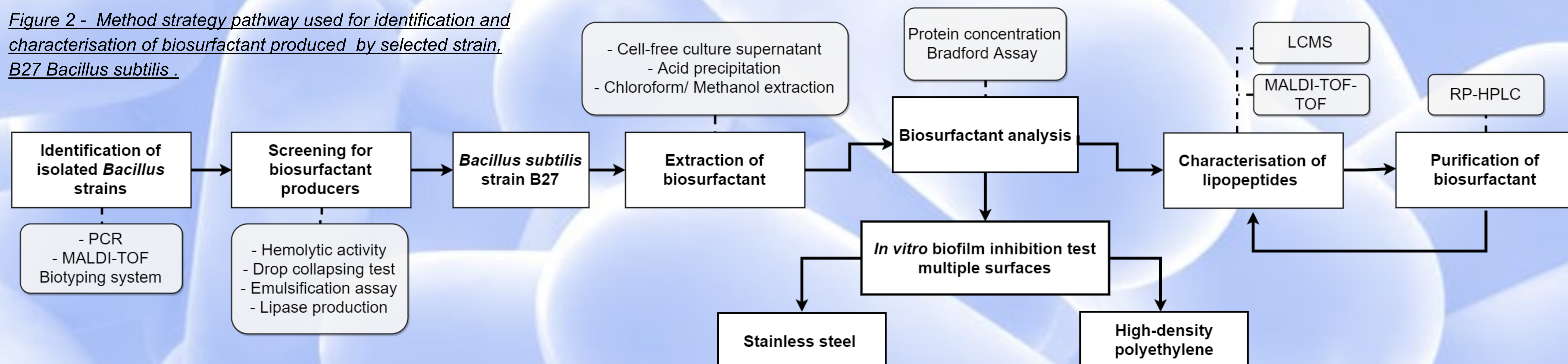
Figure 1 - Showing the Biofilm formation cycle on surfaces; attachment (1), growth (2) and dispersal (3) [1]

Methods

The *Bacillus* strains were isolated from primary effluent (Deephams Sewage Treatment Facilities, Edmonton, UK) and poultry/animal waste (A.K. Wood Poultry Farm, Fold Farm Partners and Leamon Pig Farm Ltd, UK) [4].

Figure 2 shows the pathway leading to identification and characterisation of the biosurfactant produced by the selected strain B27. Biofilm inhibition tests were also performed.

Figure 2 - Method strategy pathway used for identification and characterisation of biosurfactant produced by selected strain, B27 *Bacillus subtilis*.



Results and Discussion

The crude biosurfactant demonstrated anti-biofilm properties against different bacteria including MRSA on both stainless steel and plastic surfaces. In most cases, the crude biosurfactant performed as efficiently as a commercially-available purified biosurfactant (Surfactin) and was more effective against *Enterococcus faecalis*, *Pseudomonas fluorescens* and MRSA (Table 1).

The extract produced by strain B27 was analysed using MALDI-TOF-TOF and was found to contain not only one but **four** lipopeptides: surfactin [m/z 1016–1074], fengycin [m/z 1447–1491], subtilomycin [m/z 3230] and subtilisin-A [m/z 3400] (Figure 3).

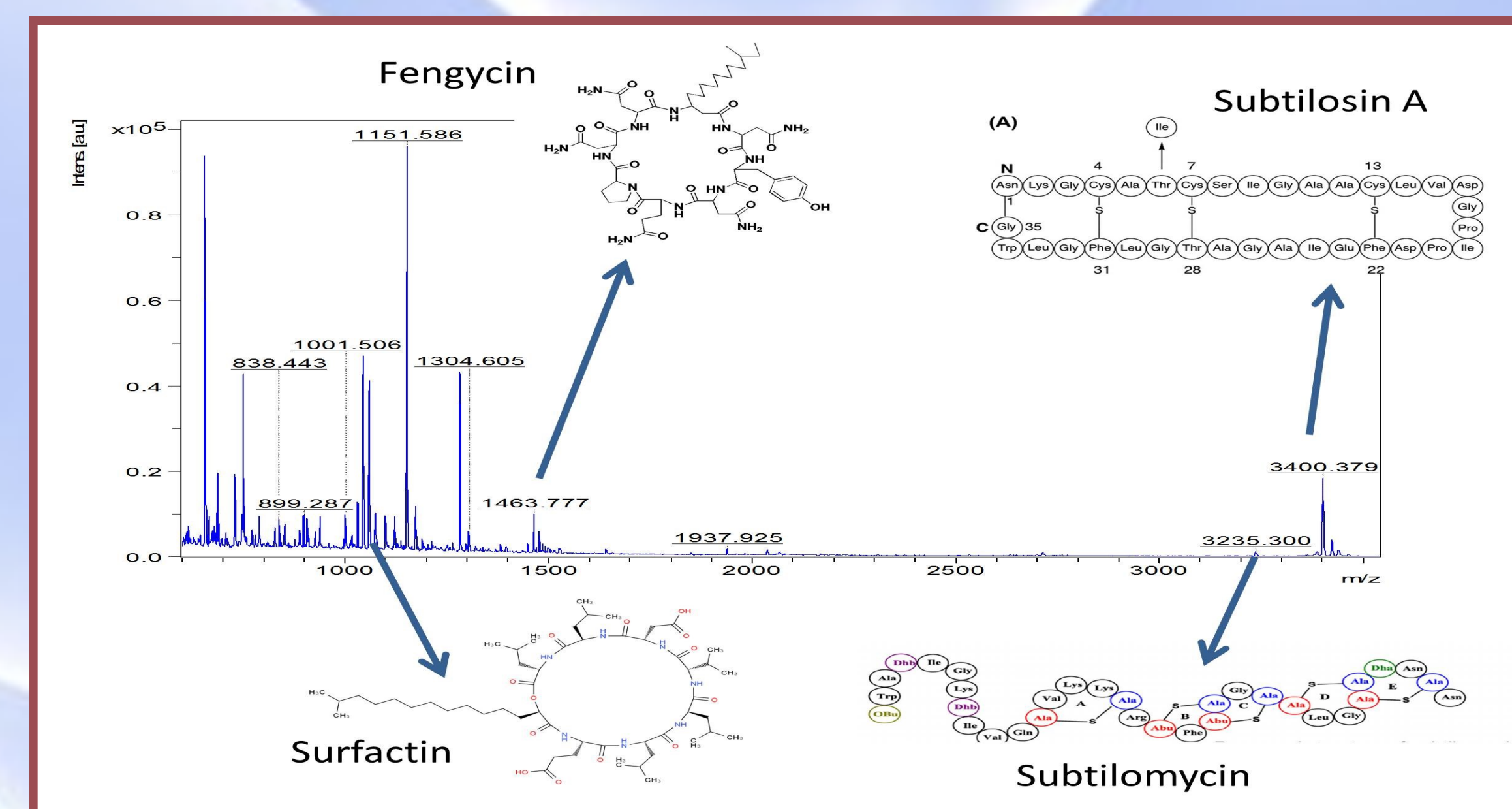


Figure 3 - Characterisation of lipopeptides produced by *Bacillus subtilis*.

Table 1a. 1b - Crude biosurfactant inhibition on Stainless steel and HD-Polyethylene surfaces; 100% biofilm inhibition, < 100% biofilm inhibition, No biofilm inhibition, NA There was no biofilm formation. Surfactin was used as positive control.

BACTERIA	SURFACE	STAINLESS STEEL		
		Control	1:10 Biosurfactant	1:100 Biosurfactant
<i>E. faecalis</i>				
<i>P. fluorescens</i>				
MRSA				
<i>S. mutans</i>				
<i>E. coli</i>				
<i>K. pneumonia</i>				
<i>S. typhimurium</i>				
<i>S. aureus</i>				
<i>S. pyogenes</i>				
<i>E. aerogenes</i>				
<i>P. aeruginosa</i>				
<i>P. mirabilis</i>				
<i>M. luteus</i>				
<i>L. monocytogenes</i>				

BACTERIA	SURFACE	HD-POLYETHYLENE		
		Control	1:10 Biosurfactant	1:100 Biosurfactant
<i>P. fluorescens</i>				
<i>S. mutans</i>				
<i>P. mirabilis</i>				
MRSA				
<i>L. monocytogenes</i>				
<i>M. luteus</i>				
<i>S. pyogenes</i>				
<i>E. aerogenes</i>				
<i>E. faecalis</i>				
<i>P. aeruginosa</i>				
<i>S. aureus</i>				
<i>S. typhimurium</i>				
<i>E. coli</i>		NA	NA	NA
<i>K. pneumoniae</i>		NA	NA	NA

Conclusion

Bacillus subtilis is a promising strain with ability of producing a number of lipopeptides able to inhibit microbial biofilms.

The biosurfactant extracted from strain B27 was highly effective against *E. faecalis*, *P. fluorescens*, MRSA and *S. mutans*.

Biofilm inhibition was more successful on stainless steel surface compared to HD-Polyethylene.

Subtilomycin production is very uncommon and has been hardly reported.

Future Work

- Further investigation of biosurfactant effects on bacterial biofilm as a bactericidal or inhibitor agent.
- Purification and analysis of lipopeptides in order to identify their specific role in biofilm disruption.
- Investigation of possible synergy effects of the different lipopeptides.

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